Background: Degradation during storage of extracted RNA may make the sample unusable for downstream assays such as reverse transcription, in vitro translation, differential display, and expression array and expression-chip analysis. It is thought that RNA degradation during storage results primarily from base hydrolysis of RNA which occurs under either low pH conditions or conditions where divalent cations catalyze hydrolysis. We investigated the effect of different storage temperatures and freeze-thaw regimens on RNA stability during storage.

Methods: RNA from a tissue sample was extracted using the Qiagen RNeasy Kit according to the manufacturer’s protocol. The concentration was adjusted to approximately 300 µg/mL using ultra-pure water. The RNA was aliquoted into single use aliquots and stored at room temperature, 4°C, or -20°C. These samples were tested at an initial time point and after 1, 2, 3, 7, 14, or 28 days of storage. Test parameters included concentration, ribosomal RNA ratio (28S:18S), and RIN number, and testing was performed in duplicate. Similarly, a subset of samples were placed at either -80°C or -20°C and subjected to up to ten freeze-thaw cycles.

Results: RNA stored at room temperature was stable through 7 days, but showed declines in all three test parameters at 14 and 28 days. No declines in RNA concentration, ribosomal RNA ratio, or RIN number were observed for RNA stored at either 4°C, or -20°C. Regardless of whether the RNA was cycled between -80°C and room temperature or -20°C and room temperature, up to 10 freeze-thaw cycles did not result in any concentration or integrity decline.

Conclusion: This study suggests that the purity of the RNA resulting from the extraction process, and the pH and composition of the RNA diluent, play the critical role in preventing degradation upon storage. When pure RNA is stored in an appropriate buffer, it is resistant to degradation under sub-optimal conditions of the type likely to be encountered during accidental mishandling or shipping delays.

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